FINAL REPORT

PHYSICAL LIMITATIONS OF LIFE

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Frederick D. Sisler Washington, D. C. July 15, 1967

Introduction

This final report "Physical Limitations of Life," Contract No. NASr-244, which commenced June, 1964, covers the period from June 1965 to date.

Previous reports (November, December 1964) listed and discussed specific physical boundaries within which simple life forms, such as microorganisms, could survive and/or metabolize in exotic or unfavorable environments. In the search for evidence of life on other planets besides Earth, microorganisms appear best suited to survive, of all other life forms, the extremes which are or were indigenous to other possible biospheres.

When this study was started, the planet Mars seemed to be the most logical choice to search for evidence of extra-terrestrial life. Although photographs from Mariner IV reveal the surface to be barren and pock-marked with craters, much like that of the moon, as mentioned in the November 1964 report (p. 17), there remains the possibility of simple life forms existing below the superficial surface. In such an environment, temperature extremes would not be encountered and shielding would exist from excessive ultraviolet radiation. Moreover, a subsurface ecological niche could prevent the escape of atmospheric gases and water if such compounds exist in small amounts.

If the essential elements necessary for microbial metabolism exist in the surface "soil" of Mars, there remains the problem of an energy source. Two possibilities have been considered in this study.

One is similar to the "Seebeck effect" where solar radiation or heat is converted to electrical energy by natural processes. The second is the direct conversion of infrared energy to chemical energy by microorganisms.

Since the "Seebeck effect" provides an indirect source of energy, i. e. electric current, it would be necessary that the electric current in turn produce a chemical form of energy that can be utilized by microorganisms. An example would be the electrolytic dissociation of water with the formation of hydrogen and oxygen. These gases are utilized by certain bacteria, e.g. Hydrogenomonas as an energy source. On the other hand, the infrared source provides the direct conversion of solar radiation to chemical energy which is affected by pigments of infrared utilizing microorganisms. Experimental:

A number of tests have been conducted to ascertain whether infrared energy could be utilized by microorganisms under conditions simulating a Martian microenvironment immediately below the "soil"

surface.

A Beckmen IR-5A double beam infrared spectrophotometer was the principle test instrument. A mixed flora of microorganisms previously cultured under light and anaerobic conditions were used in the tests. Prior history suggested these source cultures used infrared light as an energy source. Source materials and cultures were supplied by the American Type Culture Collection and Dr. Dale Jenkins of NASA. Representative of the genus Rhodospirillium

in the original source cultures. During the course of the tests, the cultures were transplanted at periodic intervals into sterile inorganic medium and incubated under light at approximately 25 °C.

Test cells used in the IR-5A contained windows of IRTRAN, a synthetic material which allowed passage of infrared light in the range from 2 to 16 microns, the limits of the IR-5A, and permitted the use of aqueous solutions. Two cells were employed, one for each IR beam. One cell was routinely inoculated with culture in inorganic medium. The other cell contained inorganic medium without inoculum and served as the sterile control.

In early tests, each of the two cells was fitted with a glass manometer. Several types of manometers were used including modified glass syringes and differential manometers of the Warburg type. The manometers were used to measure change in gas pressure indicating biological activity.

After the test cells were in place and manometer readings taken, the IR instrument was set at discrete wavelengths between 2 and 16 microns and left on for various periods of time. Incubation periods ranged from 1 to 48 hours. Differences in manometric readings between test and control cells were frequently observed. However, after several identical test runs, the readings were too inconsistent to be considered significant. Several factors may have been responsible for these irregularities, viz. (1) manometer and cell leakage, (2) reduced sensitivity of instrument, (3) concomitant

production and absorption of various gases; i.e. CO, H2, N2, etc.

A new, more sensitive, thermocouple was installed in the IR-5A. However, attempts to improve the performance and sensitivity of the manometers were unsuccessful. Moreover, it became apparent that the differential production and consumption of gases would tend to mask the overall biological activity. Since the test cultures were mixed, it seemed probable that some strains were producing gases while others were consuming them. Furthermore, the partial pressures of the gases were increasing, then decreasing with time of incubation, which can occur even with pure cultures.

The manometric method was then abandoned in favor of a coulombmetric technique based on the biochemical fuel cell. This latter technique provides a more sensitive means for measuring very weak responses to an external energy source such as infrared radiation. Instead of measuring gas production or consumption as an index of metabolic activity (Warburg method), the coulombmetric method using the biochemical fuel cell principle measures the flow of electrons resulting from chemical reactions produced by

The biochemical fuel cell is a device developed by the writer to demonstrate an electric phenomenon existing in the oceans which is produced by the interaction of solar radiation, photosynthesis and biochemical processes of microorganisms. This combination of physical biological and chemical forces results in a continuous electric current as long as there is a continuous or repeated source of solar energy. In this study, infrared radiation served as the external energy source. In principle, the phenomenon is similar to the Seebeck effect.

biological processes of metabolism.

The modification of the IR-5A instrument used for coulombmetric measurements was essentially as follows:

An asbestos wick saturated with KCl agar in a plastic, flexible tube was connected to one port of each IRTRAN cell (inner ports), and platinum wire electrodes were inserted thru a diaphragm enclosing a second port of each cell (outer ports). A current meter sensitive to the microampere range connecting the two platinum electrodes was used to measure electron flow between cells. The arrangement of the salt bridge and platinum electrode leads were such as to permit the unobstructed IR beam to pass through the windows of each cell.

In practice, both cells were filled with an inorganic salts medium. One cell was inoculated with a small amount of a live culture of bacteria. Using the double beam infrared as an energy source, the instrument was set at preselected wave lengths (between 2 and 16 microns with this instrument) and ampere readings observed at various time intervals, following the procedure used with the manometric method.

A number of repeat runs using the method described above were made. Readings were more consistent with the coulombmetric method in comparison with manometric technique, although the two types of readings, i. e. volume of gas change versus electric current flow, are not precisely comparable.

The results of the repeat runs were, in general, inconclusive.

However, an interesting feature was noted. The most frequent activity in terms of current output, was at or near the 3 micron wave length.

This corresponds to the wave length stretching frequency for the

O-H bond, suggesting molecular rearrangement of water molecules.

The writer feels that by refining the instrumentation and techniques described above, a very sensitive tool could be provided for measuring the infrared energy utilization of simple microorganisms, lichens and tissues of higher organisms.

Discussion:

Perhaps the most challenging concept of the civilized mind is that of the origin and destiny of life on earth and in the universe.

Much thought and speculation has been devoted to this subject. Until this generation, the idea that life may exist, particularly intelligent life, on other planets was the exclusive property of a few scientists and a few more science fiction writers. Today, space engineering has changed this picture. Everybody is interested in this subject.

We now have the capability to visit other planets, to obtain material evidence as to whether life is unique to the planet Earth, at least within our own solar system.

Beyond our solar system, the vastness of space seems to preclude direct contact with planets of other suns in the foreseeable future. For the immediate future, then, Moon and Mars are the logical targets for retrieving surface material for direct examination for fossilized or living forms of life.

The efforts of this study have been directed to the possibility of some form of simple life, such as microorganisms, existing on Mars. The Moon does not seem a likely habitat for actively metabolizing cells of any type familiar on Earth.

Since the time this study was initiated, the Mariner photos
have revealed a rather grim looking surface on Mars. No evidence
of organized life is apparent. These pictures do not preclude, however,
the possibility that life may have existed during some past age. Nor
do they rule out possible existence of former seas on the Martian
surface or vestiges of life below the immediate surface.

Looking at our own Earth, we see processes existing which, in time, could eventually dissipate all surface water. Water molecules in the ocean are split and reformed constantly. There is a delicate balance existing between the reversible reaction $H_2O = H_2 + 1/2O_2$. In our oceans, the combination of photosynthetic and microbiological processes break and reform water molecules continuously. Specific enzymes are involved in the hydrogen-liberating and the hydrogen-fixation processes. In the laboratory, it can be demonstrated that the enzymes which fix hydrogen can be blocked, permitting the hydrogen-liberating enzymes to continue to function. Under such circumstances, hydrogen from the destruction of water escapes to the atmosphere and, being the lightest of elements, eventually escapes from the atmosphere to space. In time, this process could eventually cause the

The question as to whether Mars had oceans or any substantial bodies of water is therefore of considerable interest since it poses a second question. If Mars had oceans, what caused them to vanish?

Until samples of the Martian crust are recovered and returned to Earth for examination, the geological and "paleontological" record must be reconstructed by less direct methods.

The latter include (1) continued Mariner-type flybys with improved remote sensors, (2) soft landings on the Martian surface by unmanned space vehicles equipped with probe or contact-type sensors and (3) earth-bound observations and experiments to simulate possible Martian environments. In addition, the examination of meteorites provides some clues to the chemical composition of other planets.

Most exciting from the standpoint of this study is evidence in meteorites of the existence of organic matter and water, including sea water resembling that of the Earth's oceans.

It seems highly probable that microorganisms were among the first forms of life on Earth. It is difficult to imagine higher forms of life existing prior to microorganisms without visualizing an environment highly polluted with accumulating excreta or the preserved remains of dead animals or plants.

It is also highly probable that the last surviving life on a dying planet will be microorganisms. The extreme conditions under which microorganisms can survive, presented earlier in this study, cannot be tolerated by any other higher form of life. It has been demonstrated that some higher plants and animals including lichens, certain worms,

seeds of higher plants, insects, arachnids, etc. have shown an amazing ability to adapt or survive some extreme environmental conditions such as low temperature, toxic chemicals and dessication. Considering the entire spectrum of environmental conditions which may be encountered on other planets, as well as Earth, only singly cell microorganisms or their spores (or conceivable self-restoring nuclear residues) qualify as the most resistant form of life.

It seems fairly obvious that if Mars had a biosphere on its surface similar to Earth, there must have been free water. If this was the case, most of the free water has disappeared. Under such conditions, one would expect the vestiges of remaining life to be concentrated in the residues of dried up lakes or oceans. If the Earth should lose its surface water, either as a result of some cataclysm or by gradual oxidation (loss of hydrogen), as it may now be doing, the last surviving life would most likely be microorganisms buried in the ocean beds. In such an environment, the remaining free water on the Earth's surface would be concentrated. Since the loss of surface water would also be accompanied by the loss of the Earth's atmosphere, extreme temperature fluctuations would follow and ultraviolet radiation greatly intensified on the surface. The microflora buried in the ocean floor would be protected from temperature and UV extremes for an indefinite period of time, whereas all surface biota would perish.

Conceivably, the only other form of life competing for survival with microorganisms under the conditions described above might be man himself or a highly intelligent evolutionary offshoot. Today,

man alone is unique in being able to master and control his environment to survive under extreme conditions. Man is also capable of completely destroying his race and this possibility seems to weigh heavily in favor of microorganisms as the ultimate survivors on Earth.

Returning again to Mars, a logical place to search for vestiges of life on that planet, which may have had surface water and a more favorable atmosphere, would be the microenvironments within dried lake or sea beds. This has been the rationale behind the experimental part of this study.

If life ever existed on Mars, important clues on the ultimate destiny of life on Earth may be found buried in the Martian crust.

Obviously, earth-bound experiments cannot furnish proof of the supposition stated above, only what may be possible. It remains for future contact exploration of Mars to resolve this riddle, one of the most challenging objectives of science and engineering today.

Summary and Conclusions:

A case has been presented that microorganisms may be present:
on Mars immediately below the surface crust.

An evaluation of present knowledge of the survival of microorganisms under extreme physical conditions favors these simple organisms as the logical candidates to withstand the Martian environment.

Laboratory experiments to demonstrate the utilization of infrared radiation as sole energy source for microbial metabolism under simulated subsurface Martian microenvironments were inconclusive.

The most significant observation from these tests was the apparent

alteration of the molecular structure of water by living cells, as evidenced by the activity at the approximate wave length corresponding to the O-H bond stretching frequency.

A laboratory technique was developed to measure cell metabolism by means of electrical currents generated by metabolic activity.

This technique was based on the biochemical fuel cell principle.

Compared to the conventional manometric method of the Warburg type, the coulombmetric method described in this report proved more sensitive and the readings easier to interpret. It seems particularly suitable for measuring cell response to very small external stimuli.

Further improvements in sensitivity are possible using more efficient electronic meters such as a vibrating reed electrometer.

It is concluded that further studies of the geochemistry and "paleontology" of Mars will increase our knowledge on the origin and destiny of life and the ultimate fate of the oceans and atmospehere of the planet Earth.